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Manipulating Aeroponically Grown Potatoes with Gibberellins and Calcium Nitrate

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Abstract

Maximizing tuber number and yield in an aeroponics production system is imperative to make it cost effective and to make the best use of all facilities and inputs. Manipulating the plants hormonal makeup is a strategy. GA3(GA), Calcium Nitrate(CaN) and their combination were foliarly applied several times to potato plants of cvs. Favorita and Mira during both the autumn and spring growing seasons in a semi tropical setting in Sichuan. A contrasting treatment of an anti-gibberellin(Anti) was also studied in both seasons. Significant differences in plants height, stem diameter, chlorophyll content, leaf area index, the number of stolons, the number of stolon branches and tuber production were observed among different treatments and cultivars. For the cv. Favorita, CaN + GA and GA increased tuber weight per plant by 63 and 49% in autumn and 53 and 73% in spring respectively as compared to the control(CK); tuber number per plant under CaN + GA and GA treatments increased by 90 and 85% in autumn and 35 and 52% in spring respectively. For cv. Mira, the trend was similar but not as dramatic. Tuber numbers over 1400 per square meter were harvested with cv. Mira during the spring season with the GA and CaN + GA treatments.

Resumen

La maximización del número de tubérculos y el rendimiento en un sistema de producción aeropónico es imperativo para hacerlo redituable y para hacer el mejor uso de todas las facilidades e insumos. La manipulación de la constitución hormonal es una estrategia. GA3 (GA), nitrato de calcio (CaN) y su combinación, se aplicaron foliarmente varias veces a plantas de papa de las vars. Favorita y Mira durante los ciclos de cultivo del otoño y la primavera en una instalación semi-tropical en Sichuan. También se estudió un tratamiento contrastante de una antigiberelina (Anti) en ambos ciclos. Se observaron diferencias significativas entre los tratamientos y variedades en altura de las plantas, diámetro de tallos, contenido de clorofila, índice de área foliar, número de estolones, número de ramificaciones de los estolones y producción de tubérculo. Para la variedad Favorita, CaN + GA y GA incrementaron el peso de tubérculo por planta en un 63 y 49% en el otoño, y 53 y 73% en la primavera, respectivamente, en comparación con el testigo (CK); el número de tubérculos por planta bajo los tratamientos con CaN + GA y GA aumentaron 90 y 85% en el otoño y 35 y 52% en la primavera, respectivamente. Para la variedad Mira, la tendencia fue similar, pero no tan dramática. Se cosecharon tubérculos en números superiores a 1400 por metro cuadrado de la variedad Mira durante el ciclo de cultivo de la primavera con los tratamientos GA y CaN + GA.

Keywords Gibberellins · Anti gibberellins · Calcium nitrate · Potatoes · Aeroponics

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Introduction

Potato (*Solanum tubersosum* L.) is the third most important global food crop (Hancock et al. 2013) and the fourth most important crop in China (Zheng et al. 2016). China is the world's largest potato producer since 1993 (Wang and Zhang 2004; Scott and Suarez 2012). Yield varies remarkably from region to region within China (Jansky et al. 2009). Inadequate supply of good quality seed tubers is one of primary constraints (Hermansen et al. 2012; Buckseth et al. 2016). Aeroponics is a method for

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potato mini-tubers production from micropropagated plantlets (Ritter et al. 2001; Buckseth et al. 2016). One of advantages of the aeroponic system is that it can produce a large number of relatively uniform tubers (Christie and Nichols 2003; Chang et al. 2012). In many respects the aeroponic systems is the best system for the production of pre-basic seeds of potato (Buckseth et al. 2016). To optimize aeroponic systems many horticultural management factors have been studied such as root zone temperature, pH, water stress, nitrogen supply (Oraby et al. 2015), electrical conductivities of nutrient solution (Chang et al. 2011), plant density and harvesting intervals (Farran and Mingo-Castel 2006) and nutrient interruption (Chang et al. 2008). Although aeroponics is becoming an exceptional mini-tubers production technology (Tierno et al. 2014), current mini-tubers production capacity can't meet the demand for seed (Jansky et al. 2009). Optimization of the production technology is still needed for specific environments and for specific potato cultivars (Buckseth et al. 2016).

Sichuan is one of the major potato production areas of China, characterized by its all-year round potato seasons and supply. Potato pre-basic seeds can be produced in spring and autumn seasons (Wang et al. 2017). The spring crop starts from the cold short day winter and ends in the warm long day summer, whereas the autumn crop from the warm long day summer to cool short day winter. Therefore, the two contrast growing seasons pose a challenge to potato pre-basic seed potato production. There is great potential to further increase the tuber yield and tuber number per plant under the aeroponics system. Hormone manipulation is our priority in this study to better adapt the potato to the two opposite trending growing seasons.

As one class of hormones and growth regulators (Zhang et al. 2003; Lee and Soh 2007), gibberellins plays an important role in the plant growth and development (Li et al. 2014). Although gibberellins are a well-known factor that is unfavorable for early tuberization (Menzel 1980), the most apparent and widespread effect of gibberellins is a promotion of growth by stem elongation and an increase in height (Wittwer and Bukovac 1958). Exogenous gibberellins increased the plant height, stem diameter, leaf area index and the tuber number of potato cultivar Atlantic (Qin 2006).

Calcium plays a major role in the maintenance and modulation of various cell functions (Rentel and Knight 2004; Talukdar 2012; Bergey et al. 2014), especially related to membrane structure and function and to cell wall structure (Palta 2010; Ding 2013). Exogenous calcium application using an appropriate concentration could improve the cold resistance of aeroponic potato seedlings (Yu et al. 2010). Li et al. (2015) reported that calcium postponed plants senescence and increased the potato plant growth, chlorophyll content, net photosynthetic rate and the yield of commodity tuber number. In addition, potatoes given calcium fertilization has reduced injury by bruising (Palta 2010; Liu et al. 2011) as well as extended tubers storage life (Ding 2013).

The purpose of the current study is to take advantage of the effect of gibberellins and Calcium Nitrate to regulate vegetative growth in the two contrasting seasons. Therefore, two potato cultivars with different genetic backgrounds was used to increase the yield of tubers: cv. Favorita, a tuberosum type of long day adapted variety from Northern Europe and cv. Mira, a very old stable variety well adapted to the shorter days of sub-tropical highlands of south west China.

Materials and Methods

Plant Materials and In Vitro Multiplication

Favorita and Mira plantlets were multiplied in vitro on standard MS medium. The temperature in the growth room was approximately 23 °C, photoperiod was 16 h, and the light was supplied by Philips Light Emitting Diode at 3690 lx.

Hydroponic Seedlings

Uniform 25-day-old tissue culture plantlets without roots were transplanted to 3×3 cm polyfoam board and were kept in hydroponic nutrient solution for 20~25 days in the glasshouse before being transplanted to the aeroponic system with attached roots. The temperature in the glass house was maintained between 20 and 25 °C, with a relative humidity between 50 and 63%.

Site Description and Aeroponic Systems

Both the autumn 2015, spring 2016 experiments were conducted at the Crop Research Institute, Chengdu, Sichuan Province, Southwest China (30°66 N, 103°54 E, 500 m a.s.l.). The average temperature, day length and solar radiation data are presented in Fig. 1. Experiments were carried out under these climatic conditions in a semi controlled greenhouse. Heating was only done at the beginning of the Spring season experiment. Fans were used for cooling. A shade cloth that can be automatically rolled over the greenhouse not only provide cooling effect but also reduced light entry by 33%. This was only done in the afternoons on very hot sunny days late into the spring crop growing season.

Plant roots were misted for 30 s every 10 min with an improved and modified Solution II (Wang et al. 2017). The pH and EC of the nutrient solution were maintained 5.5~5.8 and 3.0 respectively during the experiment. Nutrient solution was replaced weekly. The macronutrient concentration of

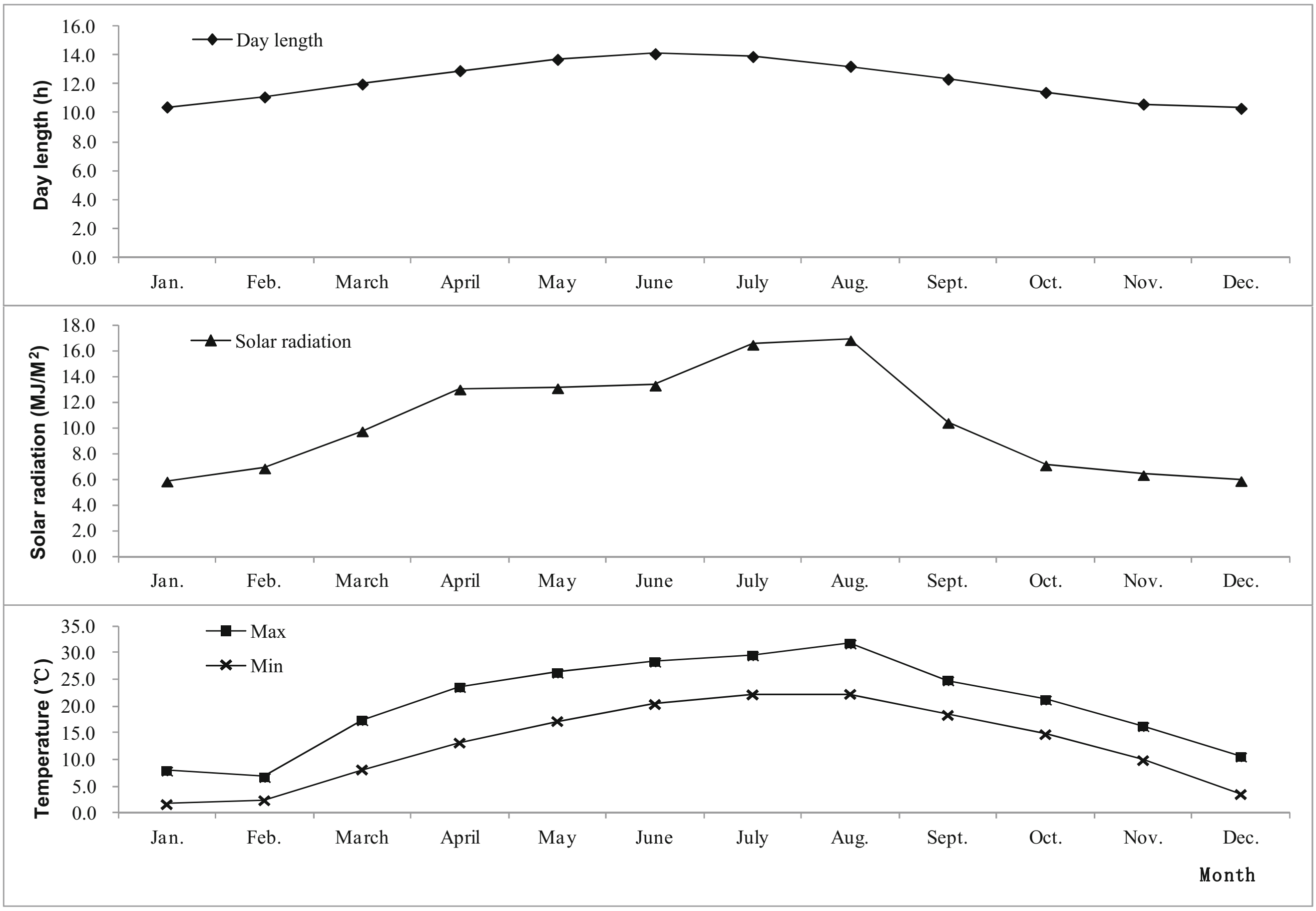

Fig. 1 Monthly average temperatures, day length and solar radiation in Chengdu plains of Sichuan Province between 2011 and 2013

nutrient solution was 1.25 mmol l^{-1} NH_4NO_3, 1.80 mmol l^{-1} KH_2PO_4, 8.85 mmol l^{-1} KNO_3, 1.40 mmol l^{-1} $MgSO_4{\cdot}7H_2O$ and 1.60 mmol l^{-1} $Ca(NO_3){\cdot}4H_2O$.

Chemical Reagents and the Method of Spraying

CaN is Calcium Nitrate Tetrahydrate ($Ca(NO_3)_2{\cdot}4H_20$) produced by Chengdu Kelong chemical. GA3 was also made by Chengdu Kelong chemical. The Anti-gibberellin utilized was a wettable powder, with the trade name of Uniconazole, produced by Sichuan Lanyue Science & Technology.

All the chemical reagents treatments were sprayed early in the morning when the plants were actively growing. Uniconazole and Calcium Nitrate Tetrahydrate were dissolved and in a fixed volume of pure water. GA3 was dissolved first in small amounts of alcohol and then into a fixed volume of pure water. At the early, middle and the late growing periods, the volume of the solution sprayed was 62.5 ml m^{-2}, 130.0 ml m^{-2} and 187.5 ml m^{-2} respectively. A cardboard border was placed between treatments while spraying to avoid drifting to neighbouring treatments. The application timing of the GA, Uni, CaN and their combinations are given in Table 1.

Experimental Design and Variables Measured

A randomized block design with four replications was used with treatment size of 1.2 m^2 (0.6 m× 2 m) with 65 plants. Morphological parameters such as plant height, root length, stem diameter, the number of stolons and the number of stolon branches were recorded at 30, 45, 56 DAT in autumn 2015 and 42, 56, 84 DAT in spring 2016 respectively on a subset of 5 plants for each treatment. Chlorophyll content was measured with a Minolta SPAD-502 chlorophyll meter on the first fully matured leaf from the apex of the shoot. Five leaves were measured, averaged to a single SPAD value. Mini-tubers >3 g were harvested once every two weeks. Tubers >0.5 g were included in the last harvest.

Statistical Analysis

Statistical analysis of all the recorded data was performed by the SPSS 19.0 program. The interaction effects between cultivars and treatments were included in the analysis. When F was significant at $P \leq 0.05$, treatment means were separated by Duncan's multiple range tests.

Table 1 Details of the six treatments during two seasons

Treatments	Concentration	Timing of application	
		Autumn	Spring
CK	Pure water	Spray water	
CaN	0.5 g l^{-1}	Start to spray at 14 DAT, weekly, 5 times (14–42 DAT)	Start to spray at 28 DAT, weekly, 8 times (28–77 DAT).
GA	10.0 mg l^{-1}		Start to spray at 28 DAT, weekly, 4 times (28–49 DAT).
Uni	5.0 mg l^{-1}		Start to spray at 56 DAT, weekly, 4 times (56–77 DAT).
CaN+GA	The combination of treatments of CaN and GA		
CaN+ Uni	The combination of treatments of CaN and Uni		

CK, Control; *GA*, GA3; *CaN*, Calcium Nitrate; *Uni*, Anti-gibberellins as Uniconazole

Results

Plant Growth and Height

Plant growth was dramatically affected by the treatments in both seasons (Fig. 2). The GA and CaN+GA treatments always had significantly larger plants in both seasons for both cultivars (Fig. 2). Plant height was greatly increased by GA for cv. Mira and to a lesser degree with Cv. Favorita (Fig. 3). The CaN+GA treatment produced the tallest plants. There was no significant difference between GA and CaN+GA treatments, except for cv. Favorita during autumn season. Plants were shortest with Uni and CaN+ Uni treatments compared to the CK.

Root Length and Stem Diameter

Overall root length was significantly reduced for Uni and CaN+ Uni treatments with cv. Mira in the autumn season and to a lesser extent in the spring season (Fig. 4). The difference between cultivars was not significant.

Stem diameter for cv. Mira was significantly larger with GA and CaN+GA treatments (Fig. 5). The stem diameter with Uni and CaN+ Uni treatments were the least. Stem diameter was not significantly improved with CaN treatments as compared to the CK. Root diameter was only slightly increased in the Autumn season due to warmer early season temperatures.

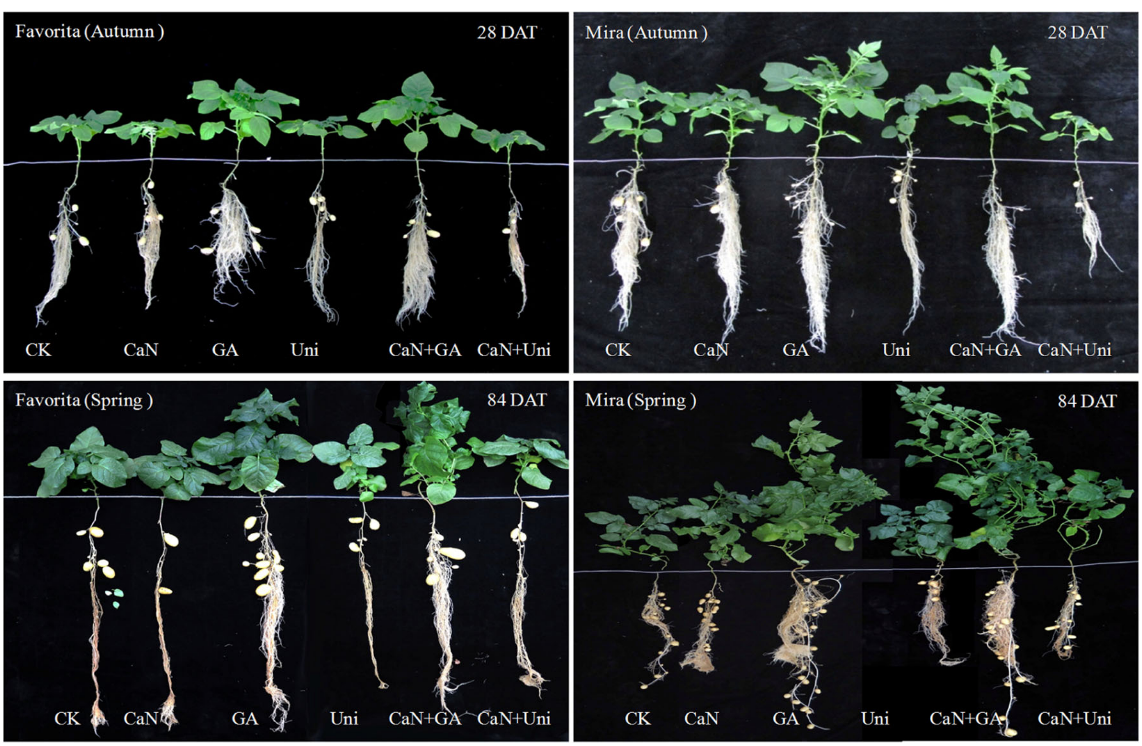

Fig. 2 Plant growth response to different treatments for cvs. Favorita and Mira in two growing seasons

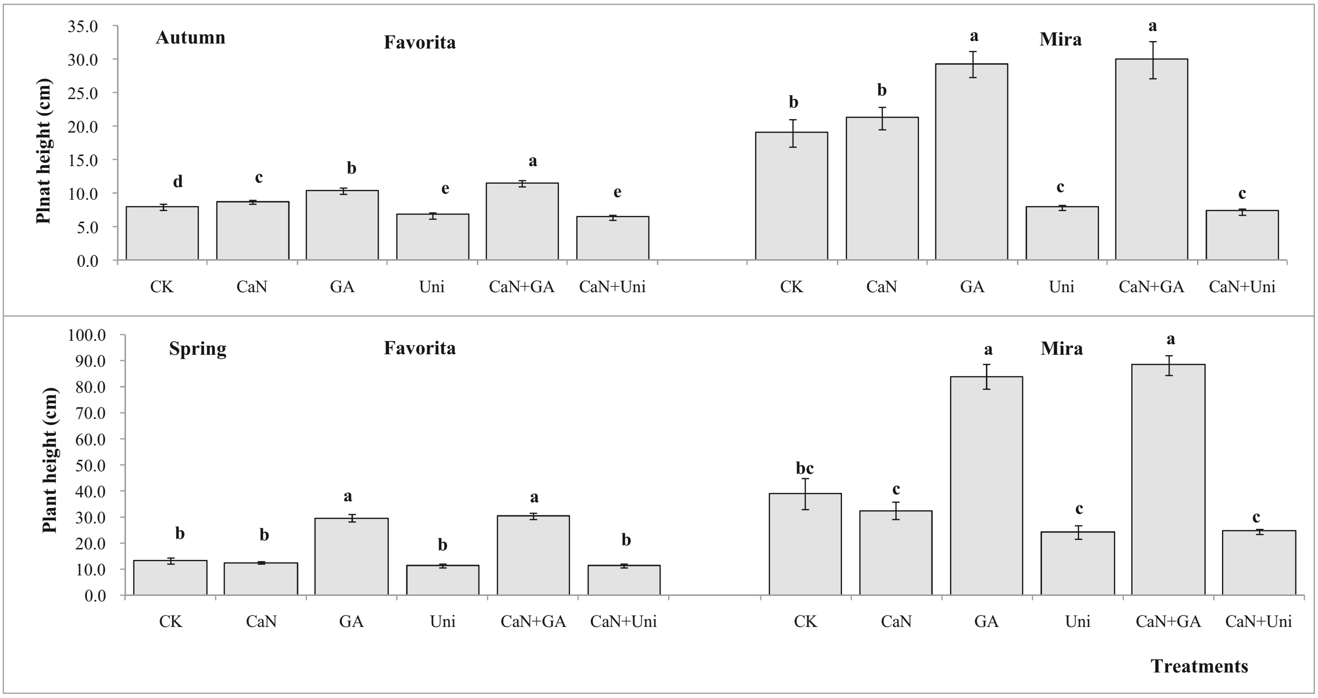

Fig. 3 Plant height at 60 DAT (Autumn) and 84 DAT (Spring) for the cvs. Favorita and Mira. *For each season and each cultivar, values followed by the same letter are not significantly different ($P > 0.05$)

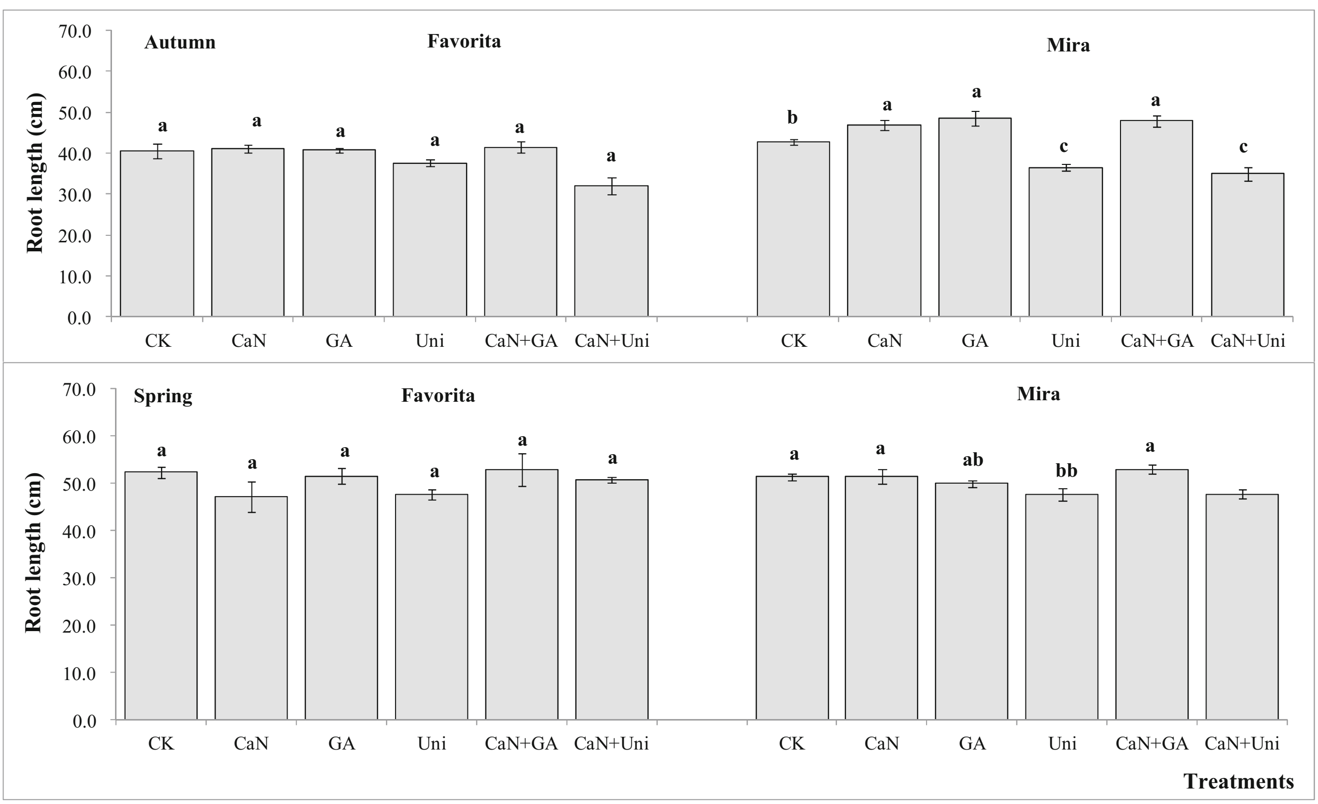

Fig. 4 Root length at 60 DAT (Autumn) and 84 DAT (Spring) for the cvs. Favorita and Mira. *For each season and each cultivar, values followed by the same letter are not significantly different ($P > 0.05$)

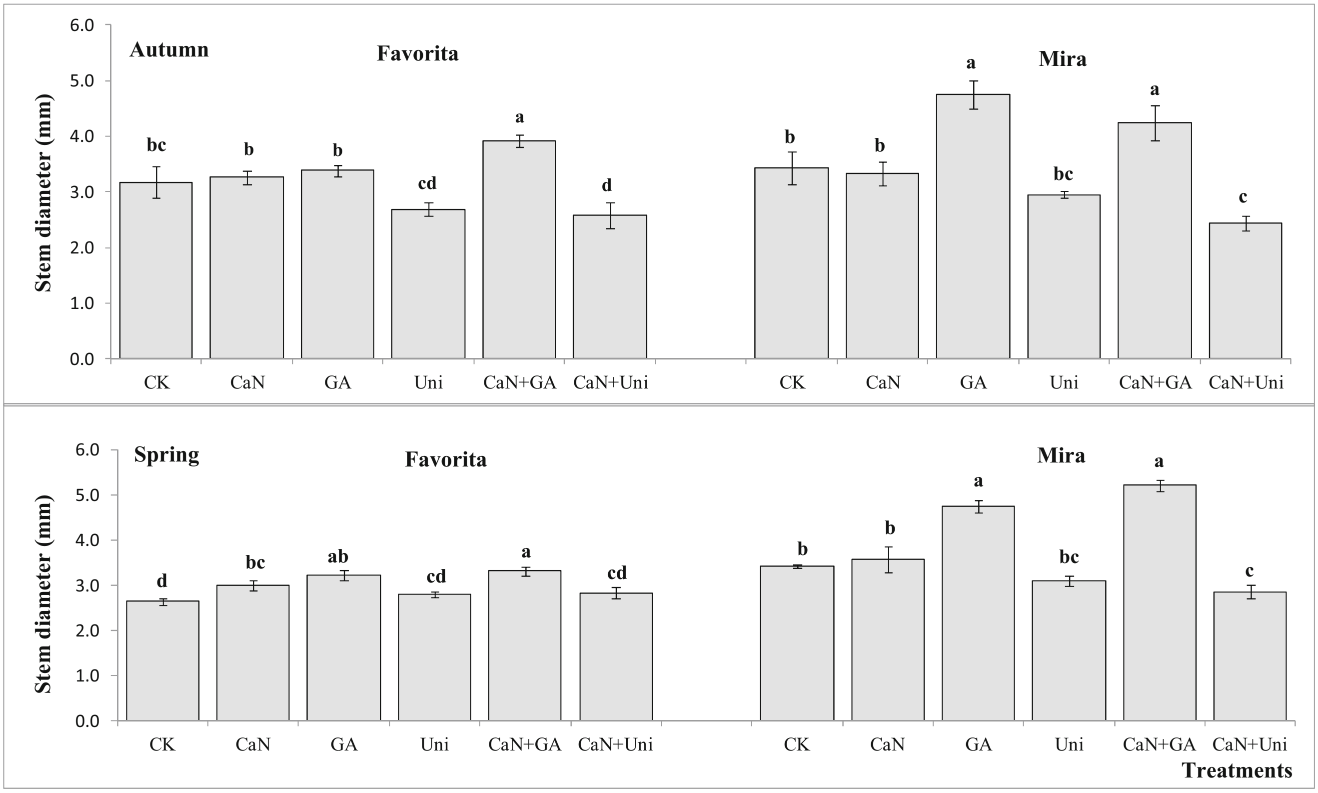

Fig. 5 Stem diameter at 60 DAT (Autumn) and 84 DAT (Spring) for the cvs. Favorita and Mira. *For each season and each cultivar, values followed by the same letter are not significantly different ($P > 0.05$)

Chlorophyll Content

Leaf chlorophyll content was significantly reduced by all hormone treatments (Table 2). The highest levels were observed in the CK and CaN treatments. Both the GA and the Uni treatments alone or in combination with CaN had a negative effect on the SPAD readings indicating a lighter color. Cv. Mira tended to have overall slightly higher SPAD readings.

Table 2 Chlorophyll content (SPAD) for cvs. Favorita and Mira in two growing seasons

Treatments	Autumn				Spring			
	Favorita		Mira		Favorita		Mira	
	45 d	60 d	45 d	60 d	56 d	84 d	56 d	84d
CK	47.0a	42.3a	50.4a	47.4b	41.4a	36.9a	40.7a	43.4a
CaN	44.5a	45.4a	51.0a	50.5a	40.5a	37.3a	41.5a	41.8ab
GA	30.9b	34.5bc	35.7b	40.5c	30.7b	35.7a	28.7b	37.5c
CaN+GA	32.2b	32.1c	35.7b	39.0c	29.8b	37.0a	29.1b	39.3bc
Uni	32.4b	37.8b	47.6a	44.9b	40.9a	36.1a	41.3a	38.4c
CaN+ Uni	32.1b	36.5b	48.3a	46.6b	41.1a	36.6a	40.8a	38.9bc

For each column, values followed by the same letter are not significantly different ($P > 0.05$)

Leaf Area

In both growing seasons, the leaf area was much larger with GA and CaN+GA treatments for both cultivars (Table 3). The differences increased as the season progressed. Generally, CaN plus GA produced the largest leaf area as compared to GA alone for cv. Favorita.

The Number of Stolons and Stolon Branches

The primary y axis denotes the number of stolons (Fig. 6). The polyline and secondary y axis represent the number of stolon branches. In the autumn season cv. Mira did not show a significant response to any hormone or CaN treatment while for cv. Favorita, stolon number was only significantly reduced with the Uni plus CaN treatment (Fig. 6). In the autumn, cv. Favorita produced an average of 10.6 stolons per plant and the cv. Mira had 9.8 per plant. In the spring, the cv. Favorita produced an average 7.2 stolons per plant and the cv. Mira 8.1 per plant. For cv. Favorita, in the spring, GA and GA plus CaN significantly increased the stolon number.

Table 3 Leaf area (cm^2) for cvs. Favorita and Mira on in two growing seasons

Treatments	Autumn				Spring			
	Favorita		Mira		Favorita		Mira	
	30 d	60 d	30 d	60 d	56 d	84 d	56 d	84 d
CK	26.8b	27.4bc	16.5d	38.9b	30.5b	41.8c	39.9c	49.6d
CaN	29.2b	26.2bc	17.1cd	37.4b	19.0c	38.1c	68.7a	59.4c
GA	47.5a	41.3b	25.2a	72.9a	55.3a	52.3b	76.9a	76.8b
CaN+GA	52.7a	63.3a	23.6ab	68.3a	59.3a	57.8a	75.2a	82.7a
Uni	30.5b	26.2bc	22.2abc	19.4c	24.4bc	40.6c	52.7b	55.5c
CaN+ Uni	22.8b	22.7c	19.1bcd	18.6c	30.1b	38.5c	71.3a	57.7c

In each column, values followed by the same letter are not significantly different (P > 0.05)

The polyline clearly shows the number of stolon's branches for cv.Mira was significantly greater and more responsive to GA than for cv. Favorita, especially in spring. In autumn, cv. Favorita's stolon branches were most with CaN+GA with 7.6 per plant, followed by GA with 5.7 per plant. For cv.Mira, the highest number of stolon's branches was 22.9 and 17.4 per plant for cv. Favorita and 62.0 and 72.5 per plant for cv. Mira with GA and CaN+GA treatments respectively.

Leaf Area Index (Spring 2016)

Leaf area index (LAI) values are shown (Fig. 7). LAI values increased gradually from 28 to 84 DAT except for the Uni and CaN+ Uni treatments of cv. Favorita. For cv. Mira, the LAI values peaked at 5.14 at 84 DAT with GA, while for cv. Favorita, and the LAI values peaked at 2.78 at 84 DAT with CaN+GA. Generally, the values of cv. Mira were greater than for cv. Favorita for all treatments.

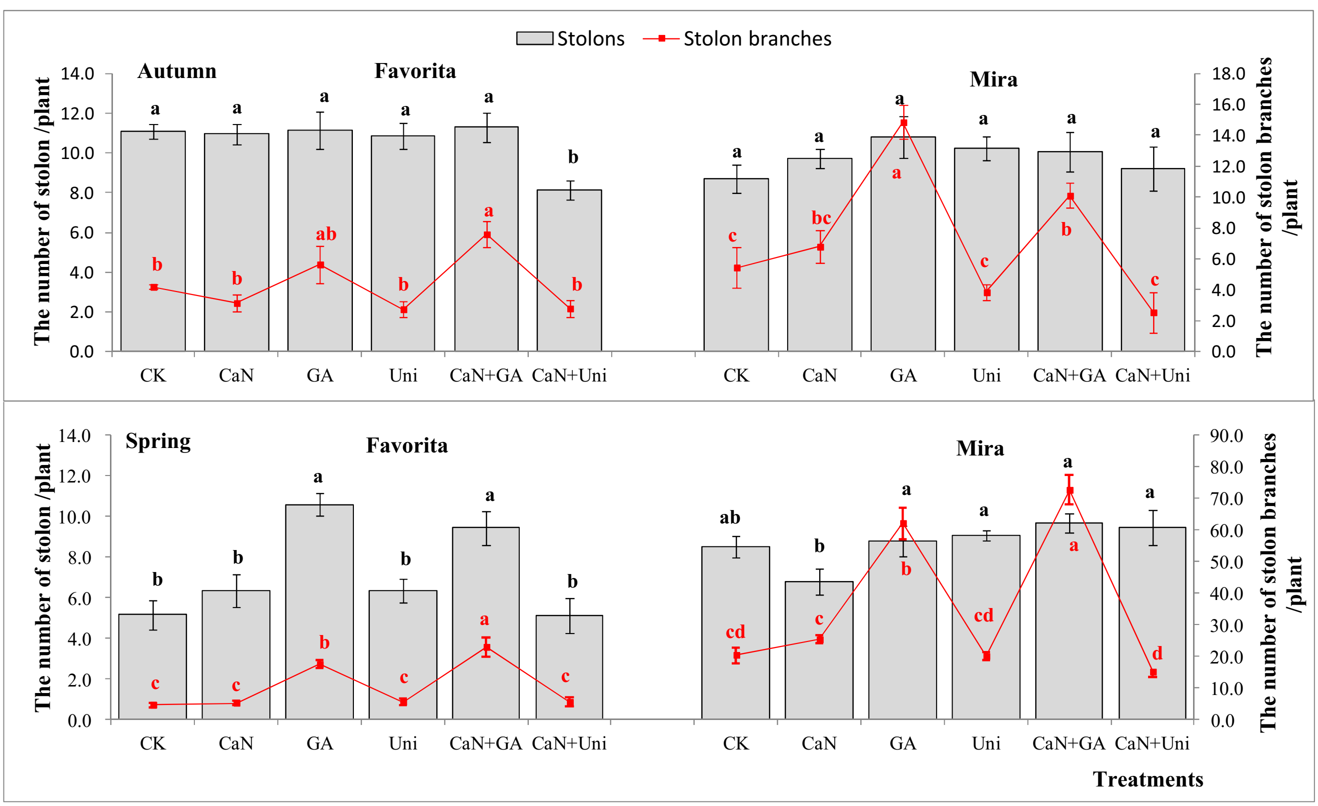

Fig. 6 The number of stolons and stolon branches at 60 DAT (Autumn) and 84 DAT (Spring) and for the cvs. Favorita and Mira. *In each season for each cultivar, values followed by the same letter are not significantly different ($P > 0.05$)

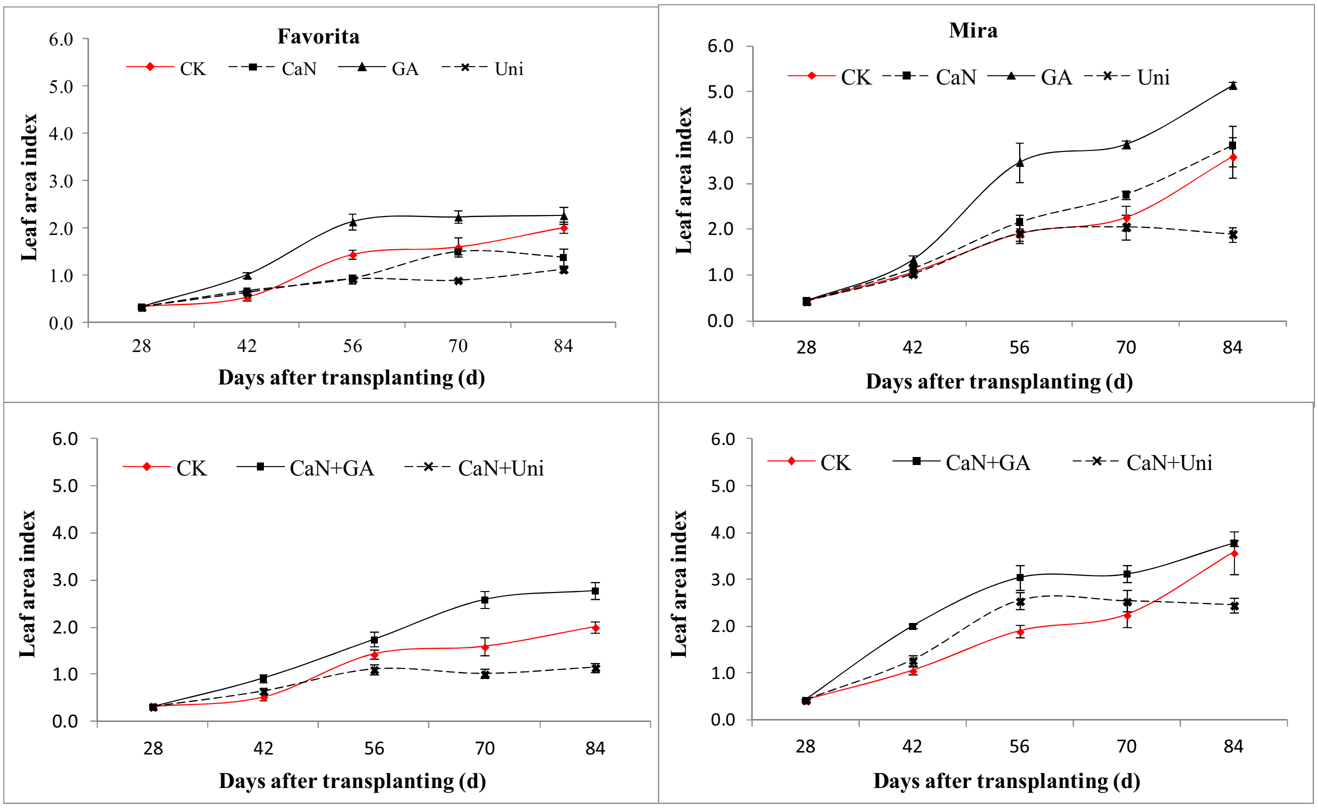

Fig. 7 Leaf area index at different DAT for different treatments in spring

Tuber Number and Yield on a per Plant Basis and per Square Meter of Aeroponic Bed

The tuber weight and number per plant were greatest for the spring crop for both cultivars (Table 4). GA and CaN+GA significantly increased the weight and tuber number for both cultivars. Cv. Mira produced over 26 tubers per plant during the spring season for these 2 treatments. This was almost double the number produced by cv. Favorita in the same season. In the autumn season cv. Mira had much lower tuber

Table 4 Tuber weight and number per plant for different treatments in two growing seasons

Cultivars	Treatments	Tuber weight (g plant^{-1})		Tuber number plant^{-1}	
		Autumn	Spring	Autumn	Spring
Favorita	CK	20.0c	33.1c	7.2c	9.4c
	CaN	21.4c	32.3c	8.4b	10.0c
	GA	29.8b	57.4a	13.3a	14.3a
	CN + GA	32.6a	50.8b	13.7a	12.7b
	Uni	11.1d	31.1c	5.1d	9.0c
	CaN+ Uni	9.8d	28.6c	4.8d	8.8c
Average		20.8	38.9	8.7	10.7
Mira	CK	41.3b	55.6b	13.2b	20.2b
	CaN	39.9b	52.0b	12.6b	16.2b
	GA	62.4a	63.3a	16.1a	26.2a
	CaN+GA	63.4a	70.1a	17.7a	26.8a
	Uni	16.3c	50.9b	7.6c	18.5b
	CaN+ Uni	14.3c	47.8b	6.3c	17.0b
Average		39.6	56.6	12.2	20.8

For each cultivar and in each column, values followed by the same letter are not significantly different (P > 0.05) numbers per plant for the 2 best treatments of 16.1 to 17.7. Meanwhile cv. Favorita had similar production during both seasons. Uni and CaN+ Uni treatments reduced the yields per plant significantly in both seasons. Tuber yield per square meter follows the same trend as the per plant data (Table 5). It is of interest to note the number of tubers produced per square meter of aeroponic bed. The best treatments (GA and GA plus CaN) produced over 1400 tubers and over 3400 g per square meter with cv. Mira in the Spring season.

Growing Period

Both GA and CaN+GA treatments prolonged the growing period, followed by CK and CaN treatments and Uni and CaN+ Uni treatments (Table 6). Uni and CaN+ Uni slightly reduced the growing period. The growing period for CaN+GA treatment was longer than GA treatment by 7 d. There was no difference between CK and CaN treatments. Cv. Mira was later maturing than cv. Favorita. The extra growing period helped cv. Mira produce more tuber number and weight.

Discussion

Potato tuberization is controlled by external factors (light and temperature), internal factors (hormones) and genotype (Demagante and Vander Zaag 1988; Vreugdenhil and Sergeeva 1999; Oraby et al. 2015). Exogenous hormones can overcome endogenous hormones and alter the plant growth and tuber formation (Lv et al. 2006). When GA at 10 mg kg^{-1} was daubed on leaves surface at 14 DAT, the content of IAA and JA in leaves increased (Qin and Wang 2006). Qin (2006) indicated that GA at 10 mg kg^{-1} increased the cultivar Atlantic's tuber number per plant. In our study, GA and CaN+GA treatments had much higher tuber number and tuber weight per plant values for cv. Favorita in both seasons. Values were from 35% to 90% greater as compared to the CK. For Mira, the trend was similar but not as dramatic. Menzel (1980, 1985) reported that a single application of GA at the start of his experiment suppressed tuber production after 4 weeks when he terminated his trial. We saw a similar trend early in our experiments. The increased tuber yield in our study with GA was related to greater early vegetative growth (taller plant, stronger stems, bigger leaf area index and more stolon branches) which created a larger factory to produce photosynthate for tuber production. GA significantly changed the above ground part of the plants. GA significantly increased the number of stolon branches. Exogenously applied GA inhibited tuber formation in the study of Vreugdenhil and Sergeeva (1999). In our study, increasing the content of GA was advantageous for stolon branches formation. The average number of stolon branches was 62.0 and 72.5 with the GA and CaN+GA treatments for cv. Mira in spring, which was much greater than for other treatments. Tuber yield per plant and per square meter with GA and CaN+GA were higher than other treatments in our study. Cv. Favorita produced fewer tubers than cv. Mira in both seasons. That agrees with Buckseth et al. (2016), who reported that a determinate growth habit cultivar tends to produce less than cultivars that are adapted to shorter day conditions such as cv. Mira. Even though yields in autumn were lower than those in spring, the positive effects of GA were prominent.

Uni inhibits GA biosynthesis, interferes with GA action, enhances GA destruction and causes shorter internodes of many plants (Ninnemann et al. 1964). With the Uni treatment in our study, tuber yield components were lowest as plant growth was severely reduced compared to the other

Table 5 Tuber weight and number per square meter for different treatments for two growing seasons

Cultivars	Treatments	Tuber weight (g m^{-2})		Tuber number m^{-2}	
		Autumn	Spring	Autumn	Spring
Favorita	CK	1080c	1787c	386c	507c
	CaN	1154c	1743c	451b	539c
	GA	1608b	3099a	718a	771a
	CaN+GA	1761a	2744b	741a	683b
	Uni	600d	168 c	277d	484c
	CaN+ Uni	531d	1545c	257d	473c
Average		1122	2100	472	576
Mira	CK	2228b	3005b	710b	1089b
	CaN	2156b	2806b	679b	875b
	GA	3349a	3416a	870a	1414a
	CaN+GA	3422a	3787a	956a	1445a
	Uni	880c	2751b	411c	999b
	CaN+ Uni	771c	2579b	339c	917b
Average		2134	3057	661	1123

Under each cultivar and in each column, values followed by the same letter are not significantly different ($P > 0.05$)

Table 6 Growing period (d) for different treatments in two growing seasons

Treatments	Autumn		Spring	
	Favorita	Mira	Favorita	Mira
CK	93	106	90	100
CaN	93	110	93	102
GA	100	126	102	113
CaN+GA	106	138	108	120
Uni	66	79	84	95
CaN+ Uni	67	79	84	95

treatments. The application of Uni was likely too early in the growing season and concentration levels were too high, so that the plants were stunted and didn't get a chance to develop.

CaN application increased the plant growth and chlorophyll content as well as improved the cold resistance of aeroponic potato seedlings (Yu et al. 2010; Li et al. 2015). However, CaN could inhibit potato GA biosynthesis and influence tuber formation (Balamani et al. 1986; Poovaiah et al. 1993). CaN slightly decreased yield for cv. Favorita and had no affect on cv. Mira yield compared to GA + CaN treatment. The yield for CaN+ Uni treatment was less than Uni alone treatment. GA resulted in excessive vegetative growth and delayed tuber formation, and thus significantly prolonged the growing period (Table 6). In terms of morphological and physiological characteristics, there was a synergistic effect between GA and CaN and an antagonism between Uni and CaN in our experiments.

The current study focused on the exogenous GA and CaN so as to promote vegetative growth. The effect of GA was obvious and the results were remarkable. GA successfully increased the yield by improving the vegetative growth of plants.

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